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PROBLEM OF CONDITIONED REFLEX RESTORATION OF IMMUNOGENESIS

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D. V. Berezhegikh
 Molotov Scientific Research
 Institute of Vaccines and Sera

[Numbers in parentheses refer to author's bibliography appended.]

The problem of conditioned reflex restoration of immunogenesis has been inadequately investigated. Many research workers deny the possibility of such restoration. The restoration of immunogenesis by means of acetylcholine, described by A. A. Klimentova and I. Ya. Nchitel' (2) of P. F. Zdrodevskiy's Laboratory, has been termed by these authors an "anamnesic" reaction. They have shown that a single administration of acetylcholine made upon the completion of vaccination causes a rise in the titer of agglutinins, notwithstanding the fact that no acetylcholine was used in the course of the immunization process. Since no adequate study of the conditioned reflex restoration of immunogenesis has been made, no clear picture of the nature of the "anamnesic" reaction is available.

In our experiments acetylcholine chloride was administered subcutaneously during the entire course of the vaccination cycle. After the titers of agglutinins dropped to their initial level, the experimental animals were given a single injection of acetylcholine chloride, while the control animals were given an injection of physiological salt solution. The titer of agglutinins in the experimental animals rose sharply as a result of the single injection of acetylcholine chloride. It remained at a low level in the control animals. We are inclined to think that in the experiments of the above-mentioned authors, as well as our own, a conditioned reflex restoration of immunogenesis under the influence of acetylcholine took place.

In work by A. O. Dolin and V. N. Krylov (1), who have studied the duration and stability of reactions of conditioned reflex immunity and the processes of their extinction, the possibility of conditioned reflex restoration of immunogenesis is clearly indicated.

I. P. Pavlov (3) said that conditioned reflexes should be regarded as a mechanism of the adaptation of the organism to changes in the environment. "I venture to think that before us there is opening up in this instance an endless field for fruitful research, a second and huge part of the physiology of the nervous system, this being a system which mainly establishes relationships not between separate parts of the organism, but between the organism and its environment." (pp 28-29)

Starting from this premise, we have decided to study the nature of the "anamnesic" reaction of the restoration of immunogenesis and to trace the possibility of the restoration of immunogenesis with the aid of nonantigenic irritants which, however, have a prolonged effect coinciding with that of vaccination. For the experiments we used chinchilla rabbits, 4 to 5 months old.

The experiment was conducted on eight rabbits -- four control animals and four experimental animals.

Determination of the titer of agglutinins was used as a test in the experiment. A heated monovaccine from a Flexner dysentery No 942 "w" day-old agar culture was used as the unconditional irritant for all the animals. Physiological salt solution in doses of 2 milliliters administered subcutaneously was used as the conditioned irritant for the control group. Acetylcholine in doses of 2 milliliters in a dilution of 10^{-6} was used as a conditioned irritant for the experimental animals. The vaccine was administered subcutaneously in increasing doses seven times at 3-day intervals.

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The initial dose of the vaccine contained 100 million microbe cells per milliliter and the final dose up to 2 billion microbe cells per milliliter of a live day-old agar culture. Conditioned irritants were administered during the entire period of immunization 10 minutes before and 24 hours after the vaccination. The agglutinin level was determined before each vaccination, and 20, 30, 45, 60, and 75 days after the completion of the vaccination. After the titers of agglutinins had dropped to their initial level, all the experimental animals were given a single injection of the conditioned irritants in doses used during the period of immunization.

To determine the degree to which physiological salt solution and acetylcholine chloride became conditioned irritants in agglutinin formation, we divided each group of rabbits into two subgroups. Two rabbits of the control group which previously had been given physiological salt solution as a conditioned irritant were now given acetylcholine chloride, and 2 rabbits of the experimental group were given physiological salt solution instead of acetylcholine chloride. The remaining rabbits of both groups served for control purposes. It was found that the titers of agglutinins rose sharply in those rabbits which had earlier received acetylcholine chloride and physiological salt solution as conditioned irritants. Low titers of agglutinins were found in the rabbits in which a conditioned irritant was substituted a single time for another.

In analyzing the data of the experiments, a conclusion can be made that acetylcholine chloride and physiological salt solution in the process of prolonged multifold parenteral administrations coinciding with vaccination became conditioned irritants in the restoration of immunogenesis. The cross-method of administering conditioned irritants in this experiment helped to distinguish the conditioned reflex effect of acetylcholine chloride from its effect as a stimulating agent.

The method was somewhat altered in the second series of the experiments.

Fifteen rabbits divided into three groups were used in the experiments: the first and second groups comprised the experimental animals, the third the control animals. A heated monovaccine from a Flexner No 942 "w" day-old agar culture was used as an unconditional irritant for all the experimental animals. Different conditioned irritants were used for each of the three groups of rabbits: the first group received subcutaneously physiological salt solution in doses of 2 milliliters each; the second group, acetylcholine chloride subcutaneously in doses of 2 milliliters in a dilution of 10^{-6} ; in the case of the third or control group, skin irritation by pricking the skin with a syringe needle was applied. Prior to the beginning of the experiment blood was taken from the edge vein of the ear of all the rabbits to determine the initial titers of natural agglutinins. Thirty minutes after the blood was taken the conditioned irritant was administered.

Injections of the above-mentioned vaccine were made, at first one half hour and then 1 to 1 1/2, 2 to 2 1/2, 3 to 3 1/2, and finally 4 hours after the conditioned irritant began to take effect. The duration of the effect of the conditioned irritant was thus prolonged to 4 hours. The initial dose of the vaccine contained 100 million microbe cells per milliliter of the vaccine. It was gradually increased until it reached the number of 3 billion microbe cells in the final injection. The immunological effect was determined by the level of agglutinins following each three combined administrations of the conditioned with the unconditional irritant. Eight combined administrations of the conditioned irritant with the unconditional were made altogether in the course of a month (28 January to 28 February 1954).

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Upon the completion of the immunization cycle which coincided with the effect of the conditioned irritant, all experimental rabbits were given a rest of 30 days (28 February to 30 March 1954).

A month later, and under the same conditions, blood was taken to determine the titer of agglutinins. On the succeeding day, 2 hours after the administration of conditioned irritants, the rabbits of the first group were given subcutaneously one milliliter of physiological salt solution instead of one milliliter of antigen; each rabbit of the second group received subcutaneously 2 milliliters of acetylcholine chloride in a dilution of 10^{-6} ; pricking of the skin with a syringe needle was applied to the third group of rabbits. In addition, determinations of the titer of agglutinins were conducted every 4-10 days during the entire period of the experiment.

The comparative data of the experiments are given in Table 1 (appended).

The data obtained from the experiments show that the titer of agglutinins began to drop by the second half of the 1st month, and reached its initial level 2 months after a repeated single administration of conditioned irritants. It must be pointed out that although type conditioned reflexes may sometimes be extinguished in the course of an experimental day upon the administration of a nonreinforced conditioned irritant, in our experiment the conditioned irritant, although not reinforced by an unconditional irritant, continued to maintain the immunity reaction at a high level for a period of 2 months.

As a result of the investigations conducted, we established that the "anamnestic" reaction is nothing else but a conditioned reflex restoration of immunogenesis under the influence of acetylcholine. The conditioned reflex restoration of immunogenesis is as much a universal reaction as is the reproduction of any other activity of the organism.

Immunogenesis may be restored with the aid of nonantigenic irritants foreign to the organism, the application of which coincided with the vaccination cycle for a prolonged period of time.

Repeatedly administered small doses of vaccine combined with repeated injections of acetylcholine chloride as a conditioned irritant may be utilized in antiepidemic work to maintain immunity for a longer period of time.

[Appended table follows]

Table 1 Titers of agglutinins upon the Administration of Conditioned Irritants

Number of Fabbit	Group	Conditioned Irritant	Titers of Agglutinins			Factor by Which Ef- fect of Conditioned Reflex Is Higher Than Titer Upon Completion of Vaccination	Factor by Which the Conditioned Reflex Effect Surpasses Titer After a Month's Rest.
			Final Upon Completion of Vaccination	After a Month's Rest	Upon a Repeated Single Administration of Conditioned Irritants		
1	I	Physiolo-	1:640	1:640	1:2560	4	4
2		gical	1:1280	1:320	1:2560	2	8
3		salt	1:2560	1:160	1:2560	0	16
4		solution	1:1280	1:160	1:1280	3	8
5			1:320	1:160	1:2560	8	16
6	II	Solution	1:1280	1:320	1:2560	2	8
7		of acetyl-	1:2560	1:320	1:2560	0	8
8		choline	1:1280	1:320	1:2560	2	8
9	III	Pricking	1:80	1:160	1:1280	16	8
10		with	1:640	1:80	1:320	1	1
11		needle	1:2560	1:320	1:2560	0	16
12		of	1:2560	1:640	1:2560	0	4
13		syringe	1:2560	1:160	1:1280	1	8

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